

TABLE 1

Curve Number	U.A.-Identification	Fibrinogen Equivalent	mg/ml
Sample size (0.2 ml, 2 $\mu$ l of 1/10 dilution.			
1. (calibration)	0.25 Mg. Rabbit IgG	0.32	Calib.
2.	10 <sup>7</sup> (Fibrinogen-Fibrin-Polymer)	0.33	0.16
3.	700,000 (Fibrinogen dimer)	0.036	0.18
4.	340,000 (Fibrinogen dimer)	0.35	1.75
5.	280,000 (Fragment X)	0.027	0.14
6.	40,000 to 30,000 (Fragment A, B or C)	0.006	0.03
7. (calibration)	0.5 Mg rabbit IgG	0.16	calib.

## EXAMPLE 3

A second method for attaching a separated protein to the novel gel of this invention has been demonstrated. In this method the conventional gel filtration methods for detection of fibrin complexes by plural fraction collection and multiple analyses of the eluted protein to characterize the chromatograph is superseded.

The proteins are separated by filtration through the glyoxal agarose gel of this invention separating them into displaced positions in the gel. Subsequently, the separated displaced proteins in the gel are immobilized into tight zones by electrophoresing alkaline cyanoborohydride into the suspending gel medium. Use of small disposable columns (Pasteur pipettes) for this zonal immobilization is advantageous. Thereafter, the immobilized zonal fractions are stained. A fluorescent or radio iodinated anti-human fibrinogen antibody is useful as the staining medium.

The procedural steps of Example 3 are illustrated in FIG. 3. An analysis is depicted based on the procedure in FIG. 4.

Referring specifically to FIG. 2 the following description is pertinent:

Cascade immunoelectrophoretic analysis of the molecular weight distribution of fibrinogen related antigens in the plasma fibrinogen sample. The plasma proteins together with a fluorescent labelled ribonuclease marker were separated by electrophoresis and subse-

quently immobilized in the sample gel. Carbamylated anti-fibrinogen antibody was electrophoresed into the sample gel and then removed by reversing current. Antibody that was retained by the immobilized fibrinogen antigens in the gel was then desorbed by electrophoresing sodium dodecyl sulfate into the gel and through a spacer gel in which the SDS was removed by precipitation with potassium ion. On continuing electrophoresis, the anti-fibrinogen antibody migrated through the spacer without interference from SDS, and into a gel containing anti IgG antibodies for measurement of the anti-fibrinogen. The areas under the rockets formed by the IgG-anti IgG precipitate measures the amount of the variant forms of fibrinogen antigens in the sample gel. The quantities of antigens are expressed on the basis that 1  $\mu$ g of fibrinogen absorbs 1.6  $\mu$ g of anti-fibrinogen antibody. Peaks 1 and 7 in the illustration are from known amounts of anti-fibrinogen antibody applied as calibration standards. The molecular weight, concentrations, and probable nature of the fibrinogen derivatives in the sample are tabulated on page 10.

Referring specifically to FIG. 4, fibrin complexes and fibrinogen in a human plasma sample were separated on a column of 4% glyoxal agarose equilibrated with rabbit fibrinogen. The protein was then immobilized, and the distribution of fibrinogen related antigens was established with fluoresceinated anti-human fibrinogen antibody.

As used herein the term glyoxal agarose is equivalent to the term glyoxyl agarose.

Having thus described the best mode presently known to me to prepare the novel gel of this invention and illustrated novel methods for its usefulness in the arts, what I claim is:

1. Glyoxal agarose.

2. Glyoxal agarose wherein the agarose molecule contains not less than 0.5 nor more than two functional units per biose unit of agarose.

3. Glyoxal agarose having a protein binding capacity under alkaline conditions of not less than about 2 and not more than about 4 mg. protein per mg. of the agarose compound.

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